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GENETIC INVESTIGATIONS ON STRIPED BASS (*MORONE SAXATILIS*) IN THE CANADIAN MARITIME PROVINCES

by

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Abstract

The striped bass (*Morone saxatilis*) is an anadromous fish native to the eastern coast of North America. Analysis of mtDNA restricted fragment length polymorphisms (RFLPs) has shown that the Bay of Fundy and the southern Gulf of St. Lawrence each contain a genetically distinct stock of striped bass. Juvenile bass from two neighboring rivers in the southern Gulf of St. Lawrence (Miramichi and Tabusintac) were not differentiated by this mtDNA RFLP analysis. No evidence of mixing was observed between the Bay of Fundy and southern Gulf of St. Lawrence striped bass populations. A second analysis of mtDNA from Bay of Fundy fish has shown that striped bass in the Saint John River are composed largely of American fish from the US coastal migratory stock. Studies in the southern Gulf of St. Lawrence have shown that young-of-the-year (YOY) striped bass from the Miramichi estuary are migratory. During the late summer of 1997 and 1998, YOY striped bass extended their range beyond the Miramichi Estuary to at least the Richibucto Estuary in the south. The magnitude of the range extension to the north was not investigated. Plankton sampling and beach seining in the Kouchibouguac and Richibucto Rivers in 1997 and 1998 failed to find any evidence of striped bass spawning. A microsatellite analysis of nuclear DNA is underway to determine if YOY collected in the Kouchibouguac and Richibucto estuaries are emigrants from the Miramichi River. This study will also serve to confirm previous conclusions regarding the level of divergence between Bay of Fundy and southern Gulf of St. Lawrence striped bass stocks. At this time there is evidence of only 2 spawning populations of striped bass in Canada: the Miramichi River in the southern Gulf of St. Lawrence and the Shubenacadie River System in the Bay of Fundy. Although the Miramichi River may be the only striped bass spawning site in the southern Gulf, many other rivers and estuaries in the area may act as important rearing habitat or "nursery areas" for YOY migrants.

Résumé

Le bar rayé (*Morone saxatilis*) est un poisson anadrome indigène de la côte est de l'Amérique du Nord. L'analyse du polymorphisme de restriction de l'ADN mitochondrial a montré que la baie de Fundy et la partie sud du golfe du Saint-Laurent abritaient des stocks distincts de bar rayé. Des individus juvéniles de deux rivières voisines du sud du golfe du Saint-Laurent (Miramichi et Tabusintac) n'ont pu être distingués par cette méthode. Aucun indice de mélange n'a été noté pour les populations de la baie de Fundy et du sud du golfe du Saint-Laurent. Une deuxième analyse de l'ADN mitochondrial des poissons de la baie de Fundy a montré que les bars rayés de la rivière Saint-Jean étaient surtout des poissons du stock migrateur côtier des États-Unis. Des études portant sur la partie sud du golfe du Saint-Laurent ont montré que les bars rayé jeunes de l'année de l'estuaire de la Miramichi étaient migrateurs. Vers la fin de l'été, en 1997 et 1998, les jeunes de l'année élargissaient leur aire au-delà de l'estuaire de la Miramichi et atteignaient au moins celui de la Richibucto, au sud. La portée de l'agrandissement de l'aire vers le nord n'a pas été étudiée. Des prélèvements de plancton et des pêches à la senne de rivage dans les rivières Kouchibouguac et Richibucto effectués en 1997 et 1998 n'ont pas permis de trouver des indices du frai de bars rayés. Une analyse de l'ADN nucléaire microsatellitaire est en cours afin de déterminer si les jeunes de l'année capturés dans les estuaires des rivières Kouchibouguac et Richibucto sont des immigrants en provenance de la Miramichi. Cette étude servira aussi à confirmer certaines conclusions antérieures ayant trait au niveau de divergence entre le stock de bar rayé de la baie de Fundy et celui du sud du golfe du Saint-Laurent. Actuellement, il ne semble exister que deux populations de géniteurs au Canada : celle de la rivière Miramichi dans le sud du golfe du Saint-Laurent et celle de la rivière Shubenacadie du bassin de la baie de Fundy. Bien que la Miramichi puisse être le seul lieu de frai du bar rayé du sud du Golfe, plusieurs autres rivières et estuaires de la région peuvent servir d'importants habitats de croissance pour les jeunes de l'année en migration.

Introduction

Use of genetics in population discrimination

The striped bass (*Morone saxatilis*) is an iteroparous, anadromous percoid native to the eastern coast of North America (Scott and Scott, 1988). Striped bass have ranged historically from the St. John River in Florida, USA to the St. Lawrence River in Quebec, Canada (Scott and Scott, 1988). Most of the research on striped bass has been concentrated in the center of their range, in the Chesapeake Bay, Hudson, and Delaware estuaries. Little is known concerning the phylogeography of striped bass in Canadian waters. We can safely assume that these fish began to colonize Atlantic Canada during the recession of the Wisconsin Glaciation, approximately 10,000 years ago (Brewer, 1988). Although the colonial history of striped bass in our waters is interesting from a phylogeographical perspective, it is their future in Canadian waters that concerns us as biologists. Recent declines in the abundance of striped bass in the Maritime Provinces have raised many concerns among researchers about the future of this species in the area (Bradford et al., 1999*a*; Bradford et al., 1999*b*). Tagging programs initiated in 1991 continue to provide a wealth of information on the population ecology of this species. Genetic investigations complement this population data by helping to resolve and confirm conclusions regarding the level of population segregation in southern Gulf of St. Lawrence striped bass stocks. The wide variety of genetic techniques that are currently available may help us to shed some light on the population genetics of striped bass in Atlantic Canada.

The terms *population* and *stock* are often used interchangeably by fisheries scientists. This can result in some confusion on behalf of the reader. For the purposes of this document, a *population* is defined as all individuals of the same species within a defined geographic location at a given time (Murphy and Willis, 1996). A *stock* is defined as a group of fish that constitute a unique genetic resource. A stock may thus be comprised of one or more separate populations.

The merits of genetic analyses have been proven in a wide variety of fisheries contexts. In spite of their usefulness, there are some disadvantages associated with these relatively new techniques. Regardless of the type of genetic analysis that is desired, they can usually only be performed by trained technicians or scientists. The start-up costs for a molecular genetics laboratory are generally very high, as are the associated costs for equipment repairs, reagents, and equipment maintenance. The various results from a genetic analysis, whether in the form of bands on a polyacrylamide gel or an electropheorogram, are usually open to some interpretation

from the reader. As a result, biases can sometimes be introduced into the results that may have dramatic effects on the final interpretations. Newly developed technologies, such as automated genetic analyzers, have helped to reduce these biases and made results from these kinds of analyses more consistent.

Investigations into the genetics of Atlantic Canadian striped bass are important for several reasons. There is little historical information on population structure of striped bass in Atlantic Canada. In light of the recent declines in abundance of striped bass, it is possible that stocking of wild or cultured fish might one day be considered to help restore extirpated populations (Cairns et al., 1999). If a stocking program for striped bass were to be initiated in the Maritime Provinces, it would be crucial that maximum levels of genetic diversity were maintained in both donor stock and in fish that were stocked into a depleted river (Bradford and Hutchings, 1999). Rigorous effort to genetically fingerprint or "type" existing striped bass populations would also provide a means to measure and monitor introgression of escaped farmed striped bass with wild fish, should this occur in the future.

Although the overall population genetics of striped bass in the Canadian Maritime Provinces remains largely unknown, several studies have been completed that have helped us to understand both the phylogeography and population structure of this important species. Investigations have been conducted on the genetics of Maritime striped bass using both mitochondrial DNA and nuclear DNA. These investigations have used a variety of techniques to differentiate between different striped bass populations in Atlantic Canada. These different techniques, the results from each study, and their associated strengths and weaknesses will comprise the main body of this paper. In the discussion, the results from these studies will be synthesized to show the informative but incomplete picture of striped bass genetics in Atlantic Canada.

Methods

Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been widely used in genetic fisheries applications. As the name "mitochondrial" implies this type of DNA is found only in the mitochondria: organelles in eukaryotic cells that are responsible for cellular respiration. Mitochondrial genomes typically range from about 17 to 18 thousand base pairs in size (Park and Moran, 1994). Fisheries geneticists have traditionally used mtDNA for population-level studies for several reasons. It is

relatively easy to isolate large amounts of purified mtDNA from animal tissues. Before PCR technologies were widely available, mtDNA analyses were the best option for many types of genetic studies. MtDNA is clonally inherited through the maternal cytoplasm, with no apparent paternal contribution and thus no recombination (Avise, 1994; Park and Moran, 1994). This mode of inheritance means that the effective population size for mtDNA is much smaller that the effective population size of the nuclear genome for a given species (Nei and Tajima, 1981). As a result, genetic drift occurs faster in the mtDNA of isolated populations than in the nuclear genomes of these same populations (Park and Moran, 1994). The term "genetic drift" refers to variations in allele frequency from one generation to the next that are due to chance alone.

It is true that genetic drift may occur faster in mtDNA than in nuclear genomes, but the mutation rates associated with mtDNA are actually very slow. The mutation rate of the mitochondrial genome is actually much slower than mutation rates in nuclear genomes, primarily because mtDNA is inherited clonally and does not undergo recombination. This slow mutation rate means that mtDNA is best used to differentiate between populations which have not recently diverged, and are greater than 100,000 years old (Angers and Bernatchez, 1998). As a result, populations that have diverged more recently than 100,000 years ago may exhibit genetic differentiation which cannot be detected through an analysis of mitochondrial DNA. Furthermore, polymorphisms found in mtDNA are not always good indicators of the overall genetic differentiation within or between populations (Pamilo and Nei, 1988). This is due to the relatively small contribution that mtDNA makes to the overall gene pool in a population (Cronin et al, 1993).

RFLP: restriction fragment length polymorphisms

RFLP (Restriction Fragment Length Polymorphisms) analysis has been used to detect genetic variation in both individuals and populations (Park and Moran, 1994). RFLP analysis uses special enzymes known as restriction endonucleases to cut or digest DNA at specific recognition sites. Once the DNA has been cut, a number of fragments remain which can be separated through standard electrophoretic techniques. Polymorphisms present in the now fragmented DNA sample are revealed as differences in the lengths of the leftover fragments. The frequencies of the fragment lengths are then used to infer genetic differences between individual animals or populations. A large number of different restriction endonucleases are commercially available, although specific applications sometimes require the synthesis of new

enzymes. RFLP analysis has been used with varying degrees of success to analyze polymorphisms in both mitochondrial and nuclear DNA (Park and Moran, 1994).

Polymerase Chain Reaction (PCR)

The polymerase chain reaction, more commonly referred to as PCR, has revolutionized molecular biology. The polymerase chain reaction is a technique that uses thermostable polymerases (enzymes which catalyze DNA replication) to amplify short DNA segments *in vitro* (Mullis et al., 1986; Wirgin and Waldman, 1994). In a typical PCR reaction, a specific DNA sequence is targeted using two DNA primers; one for each end of the target sequence. The target sequence is then heated in the presence of the two primers, DNA polymerase, and a pool of the four basic nucleotides. Upon heating, the DNA denatures, and the primers anneal to the two opposite strands of the target DNA (Wirgin and Waldman, 1994). As the sample is cooled, the DNA polymerase recognizes the annealed primers and fills in the gaps between them with the basic nucleotides. The primers are designed to anneal to the 5' end of the target sequence, so that when DNA synthesis occurs, it does so across the target sequence in a 5' to 3' direction from one primer to the other (Park and Moran 1994). This results in a copy of the original sequence. During the next heating and cooling cycle, the copy becomes an additional template for further synthesis.

During a PCR reaction, this process or "cycle" is repeated usually 30 or 40 times, each time doubling the number of copies in the sample. Millions of copies of the target DNA can be generated in a few hours, providing sufficient material for RFLP, sequencing, or electrophoretic analyses. This technique has allowed researchers to use very small amounts of initial material from which target sequences are amplified.

Nuclear DNA

The application of nuclear DNA analysis to fisheries genetics is relatively new and has gained much momentum in recent years. Nevertheless, the potential for nuclear DNA applications to population genetics has long been recognized. The nuclear genome in teleost fishes ranges from about 0.3 to 4.0 billion base pairs in size (Ohno, 1974). Although much of the nuclear genome is responsible for gene coding, there are also large regions of the genome which are non-coding. These non-coding regions are sometimes referred to as "junk DNA" and are rich in highly polymorphic sites known as VNTRs or Variable Numbers of Tandem Repeats (Carvalho and Hauser, 1994).

Three important characteristics of VNTRs make them especially well suited for genetic applications in fisheries science. First, VNTRs are non-coding, and thus under neutral selection pressure. They are therefore free to mutate, and consequently they have very high mutation rates (Carvalho and Hauser, 1994). They are also inherited in a Mendelian fashion making them ideal for population level analyses (Wright, 1993). These highly variable VNTR regions can be amplified through PCR, and thus only minute amounts of fresh or preserved tissue or blood (Approximately 0.050 g) are needed from each test animal. This type of non-lethal sampling has obvious benefits over whole animal sampling when the organisms in question are rare or endangered.

Microsatellite DNA Analysis

Microsatellites are a class of VNTR's that consist of short tandem nucleotide repeats (O'Connell and Wright, 1997). These repetitive units usually range in size from one to six base pairs and the arrays can be repeated hundreds of times. They are abundant and dispersed within the nuclear genome, and are thought to occur about once every 10 thousand base pairs in bony fish species (Wright, 1993). This would give roughly 400,000 highly polymorphic markers in the nuclear genome of an average teleost! Microsatellite loci are easily amplified from small samples of tissue or blood through PCR. These VNTR loci are characterized by very high mutation rates that quickly lead to extensive levels of polymorphism (Angers and Bernatchez, 1998), making them ideal genetic markers for many applications. These characteristics have also made microsatellite DNA analysis a very useful tool to study species which have shown low levels of variability with other types of molecular markers (Angers and Bernatchez, 1998). This may make microsatellites an ideal marker for use with striped bass, which have shown very low levels of genetic diversity with most other types of genetic markers (Diaz et al., 1997).

Microsatellite loci are normally visualized and scored manually through standard techniques such as gel or capillary electrophoresis. However with new developments in automated sequencing technologies, many laboratories are beginning to make the switch to automated genetic analyzers. These systems are both faster and more efficient than traditional electrophoretic techniques. Automated sequencers use fluorescent labels to mark nucleotide sequences, and can bypass many of the difficulties usually associated with microsatellite analyses. Slipped-strand mispairing during PCR amplification is a common source of band stuttering or "noise" observed during manual microsatellite visualization (O'Connell and Wright,

1997). This noise can cause inaccurate scoring of alleles at a particular locus by obscuring allele sizes, thus making results more difficult to interpret. With an automated sequencer however, only one primer in a primer pair need be labeled, thus circumventing any noise caused by slipped-strand mispairing during PCR. Another advantage of automated fluorescent technology is that samples can be "multiplexed" due to the variety of fluorescent dyes that are available. This allows an investigator to analyze several different loci simultaneously for a given fish, thus minimizing laboratory time and costs.

Although microsatellite DNA may indeed be an ideal molecular marking system for many types of genetic investigations, it is also a very expensive one. The isolation and synthesis of microsatellite primers is a time consuming and arduous process. Microsatellite primers labeled with fluorescent dyes are expensive to purchase as is the associated hardware and software. The costs are justified by the fact that this type of analysis is faster and far more sensitive than previously favored methods of differentiation, such as RFLP analysis of nuclear or mitochondrial DNA.

Historical Genetic Studies

I. 1991-1992 mtDNA Studies

In 1993, the first results were published from a two-year investigation into the genetics of Canadian striped bass (Wirgin et al., 1993*a*). This study represents the first effort to genetically determine the relatedness between different populations of striped bass from Canadian Rivers. Striped bass were collected in the southern Gulf of St. Lawrence (Fig. 1) from the Miramichi and Tabusintac Rivers, and Bay of Fundy striped bass were collected from the Shubenacadie River. Fish were also collected from the Hudson River and Chesapeake Bay populations of the United States to act as representatives of the American coastal migratory stock (Wirgin et al., 1993*a*). Mitochondrial DNA was isolated from these fish, cleaved with a number of restriction enzymes, and screened for polymorphisms with probes that were developed specifically for striped bass (Wirgin and Maceda, 1991). Four different mtDNA length genotypes were detected in Canadian striped bass; all of which had been previously detected in American fish (Wirgin et al., 1989, 1990). The frequencies of the different length genotypes detected were then used to infer genetic subdivision among the five populations that were sampled (Wirgin et al., 1993*a*). All three Canadian populations were shown to be genetically distinct from the American fish collected in the Hudson and Chesapeake Estuaries (Wirgin et al., 1993*a*).

Significant differences were detected between the two Gulf of St. Lawrence populations and the Shubenacadie River population (Wirgin et al., 1993*a*), indicating that the Bay of Fundy and Gulf of St. Lawrence populations were likely reproductively isolated from one another (Wirgin et al., 1993*a*). Tagging data from the Bay of Fundy supports this conclusion (Rulifson et al., 1987). Approximately 1400 fish were tagged in the Bay of Fundy and none of the 237 tag returns were from the Gulf of St. Lawrence. Mark and recapture experiments in 1984 tagged several hundred adult striped bass in the Kouchibouguac River and no recaptures were made in the Bay of Fundy or in any of its rivers (Hogans and Melvin, 1984). No significant differences in length genotype frequencies were detected between the two Gulf of St. Lawrence populations from the Miramichi and Tabusintac Rivers (Wirgin et al., 1993*a*). Several hypotheses have been extended to explain the apparent genetic homogeneity between these two populations (Wirgin et al., 1993*a*). Young striped bass from these two rivers could mix together at an early age, or the populations may have diverged too recently to develop significant differences in mtDNA profiles (Wirgin et al., 1993*a*). Recent extirpation and repopulation events could have occurred between the two rivers, resulting in similar genotype frequencies between the two systems (Wirgin et al., 1993*a*). The similar profiles between the two rivers could also be due to chance alone (Wirgin et al., 1993*a*). It is likely however that the striped bass captured in the Tabusintac River were actually fish that immigrated from the Miramichi River. Studies based on mark recapture data (Bradford et al., 1999*b*) have shown that adult striped bass occurring in the Tabusintac River in the fall were likely spawned in the Miramichi River.

It appears from this work that the Gulf of St. Lawrence and the Bay of Fundy striped bass populations are genetically distinct from one another. The lack of observed divergence between the fish sampled from the two southern Gulf of St. Lawrence rivers may mean very little due to the age class of fish that were sampled and the time at which the sampling occurred. Juvenile striped bass were sampled in the southern Gulf of St. Lawrence in the fall from commercial smelt box nets. Young age classes (age-0 and yearlings) were used for this study because it was believed at the time that these fish were non-migratory and thus indicative of any genetic signatures specific to their natal rivers. This is problematic because recent work has shown that at least some juvenile striped bass from the Miramichi River (age-0) are migratory and begin to move out of the Miramichi River during the late summer (Robinson et al., unpublished data). It is therefore possible that the fish collected from the Tabusintac River may have been of

Miramichi origin. Spawning of striped bass in the Tabusintac River has not been confirmed and is not supported by tagging studies of age 2+ striped bass in the southern Gulf of St. Lawrence (Bradford et al., 1999*b*).

II. 1995 mtDNA Studies

In 1995, the results of a two-year mtDNA study were published that attempted to determine the relative contribution of American fish to the Bay of Fundy striped bass fishery (Wirgin et al. 1995). Bay of Fundy striped bass were collected from the Saint John River and the Shubenacadie River. Reference collections of striped bass DNA from Long Island, New York were used as representatives of the American striped bass stock.

Mitochondrial DNA major length variants were determined for each fish and genotype frequencies were determined for each river (Wirgin et al., 1995). Four mtDNA length genotypes were detected with the analysis. These mtDNA length genotypes were designated as length genotypes A, B, C, and D (Wirgin et al., 1995). Striped bass from the Shubenacadie River were found to exhibit high frequencies of mtDNA major length genotype A, which is rarely seen in other striped bass populations (Wirgin et al., 1995). Thus, based on the frequency of major length genotype A, it was concluded that fish from the Shubenacadie River were genetically distinct from both Saint John River and Long Island striped bass. MtDNA genotype frequencies were found to differ significantly between the three populations, and were used in a mixture model to quantify the origin of adult fish in the Saint John and Shubenacadie Rivers.

A mixture model is a type of mixed stock analysis based on genetic or morphological data (Utter and Ryman, 1993). The Genetic Stock Identification (GSI) model, also known as the Mixed Stock Analysis (MSA) model, is most commonly used in conjunction with genetic data (Milner et al., 1985, Utter and Ryman, 1993). In this mixture model, contributory populations are first screened for informative polymorphic markers (Utter and Ryman, 1993, Wirgin et al., 1995). These markers are then used to establish genotypic or allelic frequencies that are unique to each contributory population (Wirgin et al., 1995). Once baseline data sets have been established for each contributory population, genotypic or allelic frequencies are then determined for the mixed population (Utter and Ryman, 1993). The relative contribution of each contributory population to the mixed population is then determined using Monte-Carlo χ^2 and Crittenden's least squared statistics (Wirgin et al., 1993a; Wirgin et al., 1993b). Two main assumptions are usually made with this type of MSA analysis (Utter and Ryman, 1993). First, it

is assumed that all populations that contribute to the mixed population are known. Second, it is assumed that all contributory populations are adequately sampled and accurately reflected in the baseline data (Utter and Ryman, 1993).

Two major conclusions came from this analysis. First, it was concluded that up to 97% of the adult striped bass in the Saint John River were of American origin. Second, it was determined that over 50% of the fish in the Shubenacadie River were native to that system. Based on these studies, Wirgin and his colleagues (1995) extended the hypothesis that the current Saint John River stock was composed entirely of migrant fish spawned in other rivers. Tagging data (Rulifson et al., 1987; Waldman et al., 1990) support the hypothesis that striped bass move between American and Canadian waters. In light of the unfortunate history of the Saint John River, this hypothesis seems likely. No young life stages of striped bass have been found in the Saint John River in recent years, presumably due to loss of spawning habitat as a result of the 1967 construction of the Mactaquac hydroelectric dam (Jessop, 1991).

There has been some debate in the literature regarding the validity of mtDNA studies in a phylogeographic context (Stellwag and Rulifson, 1995, Waldman and Wirgin, 1995). Regardless of the level of heritability in mtDNA length genotypes, recent comparative work with nuclear and mtDNA (Angers and Bernatchez, 1998) has suggested that mtDNA analyses may be problematic for other reasons. In all likelihood, mtDNA analysis may only be accurate for populations which have had a temporal scale of divergence sufficiently long enough to lead to population-specific mtDNA variants (Avise, Neigel, and Arnold, 1984). This means that for recently diverged populations, mtDNA analysis may only be meaningful if differences are detected. If no differences are detected, it could indicate that the populations are discrete but are too recent to have developed significant differences in mtDNA genomes (Wirgin, 1993*a*). Bernatchez (1998) proposes that mtDNA analyses may not be useful for phylogenetic studies on species that have diverged since the Pleistocene glaciations. In these cases, mtDNA analyses may lack the resolution needed to determine fine-scale phylogenetic histories, or even define population assemblages on large geographic scales (Bernatchez, 1997; Angers and Bernatchez, 1998). In these cases, mtDNA needs to be coupled with nuclear DNA analysis, which is capable of finer scale phylogenetic and phylogeographic analyses.

III. 1997 Nuclear DNA RFLP Studies

In 1997 a group of researchers used striped bass nuclear DNA to differentiate between stocks along the East Coast of North America (Diaz et al., 1997). Striped bass DNA was obtained from several USA populations and the Tabusintac and Shubenacadie River populations in the Canadian Maritime Provinces. Three single-locus nuclear DNA markers were used; PCR-RFLP assays were used to estimate allele frequencies at all three loci for each population sample (Diaz et al., 1997). This study found that moderate levels of genetic heterogeneity were present throughout the range of *M. saxatilis*. Allele frequencies were found to differ significantly between striped bass from the Tabusintac and Shubenacadie Rivers (Diaz et al., 1997). This study supports the previous conclusion based on mtDNA data that the Bay of Fundy and Gulf of St. Lawrence each contain a genetically distinct stock of striped bass.

IV. 1997-1998 Nuclear DNA Microsatellite Studies

In 1996, Kouchibouguac National Park and the Department of Fisheries and Oceans initiated a three-year striped bass spawning study. The main goal of this project was to determine the spawning status of striped bass in the waters of Kouchibouguac National Park, specifically in the Kouchibouguac River. Previous research (Hogans and Melvin, 1984) had provided some anecdotal evidence of spawning within Kouchibouguac National Park, although no eggs or larvae were collected.

Field collections of ichthyoplankton in the summer of 1996 found no evidence of striped bass spawning. In August however, large numbers of young-of-the-year striped bass began to move into the Kouchibouguac River and the Kouchibouguac Estuary (Fig. 2). These fish were assumed to have originated outside the waters of Kouchibouguac Park, from another nearby estuary such as the Miramichi or Richibucto (Robinson et al., 1998). The Miramichi River to the north is a confirmed spawning site for striped bass (Robichaud-LeBlanc et al., 1996), and the Richibucto River to the south is a suspected spawning area.

In 1997 and 1998, the spawning status of striped bass was examined in both the Richibucto and Kouchibouguac Estuaries by extensive ichthyoplankton sampling. These ichthyoplankton surveys failed to find any evidence of striped bass spawning although YOY striped bass moved into both of these systems during August of each year (Robinson, unpub. data). In both 1997 and 1998, YOY striped bass were also collected during August from coastal

locations in the southern Gulf of St. Lawrence between these estuaries (Fig. 2), indicating that a coastal migration of YOY bass was indeed occurring, and likely doing so on a yearly basis.

Although adult striped bass are known to undergo extensive migrations from their home systems (Scott and Scott, 1988), very little is known about the migratory habits of young-of-the-year fish. It has generally been held that these young fish do not migrate but remain in their home rivers for their first year of life (Scott and Scott, 1988; Waldman et al., 1990). This lack of knowledge is likely due to their small size, which makes them impractical for traditional mark and recapture tagging experiments. It is likely that previous conclusions regarding the migratory ability of YOY striped bass are based primarily on the geographic areas in which the fish are studied. Many of the estuaries in the United States which support substantial striped bass populations (such as the Hudson, Chesapeake, and Delaware Estuaries) are geographically much larger than estuaries that support striped bass in the Canadian Maritime Provinces. For example, the known range of YOY striped bass in the Hudson Estuary (from the spawning grounds to the coastal plain) is greater than 200 km in length. This distance is roughly equivalent to the coastline distance from the spawning grounds in the Northwest Miramichi River to the areas in the Richibucto River where YOY striped bass were captured in 1997 and 1998 (Robinson et al., 1998). The cruising distance for 5 to 10 cm long juvenile striped bass is approximately 6.5 to 13 km/day at 1.5 body lengths/second (R. Bradford, pers. comm.). It is thus logical to assume that YOY striped bass spawned in the Miramichi River range over hundreds of kilometers from the time of schooling in mid-July to the end of the growing season (R. Bradford pers. comm).

A nuclear DNA analysis is currently underway at the University of New Brunswick to determine the origin of YOY striped bass caught in the Richibucto River, Kouchibouguac River, and in transit between these two systems. Striped bass collected in the late summers of 1997 and 1998 from the Miramichi, Kouchibouguac, Richibucto, and Stewiacke Rivers are being used for the analysis. Young-of-the-year striped bass from the Stewiacke River will provide a genetic outgroup. For each population, five microsatellite loci are being amplified through PCR and visualized on an automated genetic analyzer. This study will also serve to confirm previous conclusions based on mtDNA regarding the distinctiveness of southern Gulf of St. Lawrence and Bay of Fundy striped bass populations. This microsatellite analysis will also provide a more accurate estimation of the level of genetic variability within the southern Gulf of St. Lawrence striped bass stock.

Discussion

Based on these studies, it seems likely that there are at least two genetically distinct populations of striped bass in the Canadian Maritime Provinces; one in the Bay of Fundy and another in the southern Gulf of St. Lawrence. All available evidence indicates that these populations do not mix and that fish do not migrate between these two areas. At present two striped bass spawning areas are known to exist in the Maritime Provinces. One spawning population of striped bass exists in the Miramichi River, and the other is native to the Shubenacadie/Stewiacke River system.

There are many other rivers in the Canadian Maritime Provinces that are historically suspected to have supported striped bass spawning. These include the Saint John, Annapolis, Kennebecasis, Richibucto, Kouchibouguac, Buctouche, and Tabusintac Rivers (Rulifson and Dadswell, 1995). Although these rivers may have supported striped bass spawning in the past, conclusions based on newly acquired data indicate that this historical view of stock structure in the Maritimes is likely incorrect. Thus, it can be concluded that the Miramichi and Shubenacadie/Stewiacke River systems have probably always been the major producers of striped bass in the Canadian Maritime Provinces.

It seems likely that if further population subdivision of striped bass exists within the southern Gulf of St. Lawrence or the Bay of Fundy, it will not be detected through further analysis of mtDNA. The results from the 1997-1998 nuclear DNA microsatellite analysis should provide more information with regards to the genetic diversity of striped bass within Canadian waters.

Although the molecular data set from the 1997-1998 nuclear DNA study is not yet complete, several important observations have developed from the field component of this investigation. First, YOY striped bass from the Miramichi River are capable of movement between river systems within the southern Gulf of St. Lawrence. These fish were captured moving out of the Miramichi River and into rivers located approximately 100 km south of Miramichi Bay. To the best of our knowledge, this is the first time that inter-riverine migrations have been observed in YOY striped bass. Second, other rivers in the southern Gulf of St. Lawrence, such as the Kouchibouguac, Richibucto, Buctouche, may act as rearing and overwintering habitat for YOY fish from the Miramichi River. This may have important conservation implications for striped bass in the southern Gulf of St. Lawrence. Rivers to the

north of the Miramichi River may also act as rearing habitat for emigrating YOY striped bass. It is currently unknown whether or not YOY fish immigrating into these rivers remain there for the winter, and if they do what their overwintering survival rates are. Third, the presence or absence of YOY striped bass from late summer on cannot be used as an indicator of spawning activity in any southern Gulf of St. Lawrence River. These young fish are capable of substantial movements, and as such are not necessarily indicative of a reproducing population within a strict geographic area.

The next question is, of course, what is the next step? Have we examined striped bass genetics in our area to the fullest extent possible or practical? The answer is no. We are at a stage where we are all wondering about the future of striped bass in the Canadian Maritime Provinces. In our opinion, the following four recommendations for future research should be considered to help us better understand the population dynamics of striped bass in Canadian waters.

First, exactly which rivers in the southern Gulf of St. Lawrence and Bay of Fundy currently support striped bass spawning? Young striped bass in the southern Gulf of St. Lawrence are capable of substantial movement, thereby negating their usefulness as indicators of spawning activity. Second, nuclear DNA studies should be continued in an effort to assess population differentiation and monitor levels of genetic diversity among known striped bass populations in eastern Canada. Third, nuclear DNA data should be coupled with mtDNA RFLP data to validate the conclusions based on previous mtDNA studies. Finally, genetic analyses should be conducted to quantify the temporal stability of mtDNA and nuclear DNA genotype signatures in known or suspected striped bass populations.

We are not suggesting by this paper that we need to suddenly direct all our available resources into intensely studying striped bass genetics. However, good fisheries management depends on solid science. Continued work on the genetics of striped bass in our area will complement the existing management strategy and provide us with a more robust understanding of the population dynamics of this species. By better understanding bass populations in the Maritimes today, we will increase our chances of better protecting our striped bass populations of tomorrow.

Research Recommendations

- Nuclear DNA studies should be continued in an effort to assess population differentiation among known and suspected striped bass populations in eastern Canada.
- Nuclear DNA data should be coupled with mtDNA RFLP data to validate conclusions based on previous mtDNA studies.
- Genetic analyses should be conducted to quantify the temporal stability of mtDNA and nuclear DNA genotypes and allelic signatures in known or suspected striped bass populations. These findings should be integrated with concurrent stock assessment data such as year class size and spawner abundance.
- Map migration routes, rearing habitat, and overwintering areas of young-of-the-year striped bass.

Management Considerations

- The presence of young-of-the-year striped bass from late summer on cannot be used as an indicator of spawning activity in a particular river.
- Known striped bass spawning areas, such as the Northwest Miramichi and Shubenacadie/Stewiacke Rivers, should be protected.
- A region wide approach to the protection of essential rearing and overwintering habitat should be implemented.
- Migratory corridors used by young-of-the-year striped bass may need to be protected from disturbance during the late summer to help maximize recruitment in rivers south of the Miramichi, such as the Richibucto and Kouchibouguac Rivers.

References

Angers, A., and L. Bernatchez. 1998. Combined use of SSM and non-SSM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. Mol. Bio. Evol. 15(2): 143-159.

Avise, J. C. 1994. Molecular Markers, Natural History and Evolution. New York: Chapman and Hall. 511 pp.

Avise, J. C., J. E. Neigel, and J. Arnold. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J. Mol. Evol. 20: 99-105.

Bernatchez, L. 1997. Mitochondrial DNA analysis confirms the existence of two glacial races of rainbow smelt *Osmerus mordax* and their reproductive isolation in the St. Lawrence River estuary (Québec, Canada). Mol. Ecol. 6: 73-83.

Bradford, R.G. and J. Hutchings. 1999. Genetic interaction between artificially-reared and wild Canadian striped bass (*Morone saxatilis*): risk and uncertainties. Department of Fisheries and Oceans Canadian Stock Assessment Secretariat Res. Doc. 99/03.

Bradford, R.G., D. Cairns, and B. Jessop. 1999*a*. Update on the status of striped bass (*Morone saxatilis*) in eastern Canada in 1998. Department of Fisheries and Oceans Canadian Stock Assessment Secretariat Res. Doc. 99/01.

Bradford, R.G., G. Chaput, S. Douglas, and J. Hayward. 1999*b*. Status of striped bass (*Morone saxatilis*) in the Gulf of St. Lawrence in 1998. Department of Fisheries and Oceans Canadian Stock Assessment Secretariat Res. Doc. 99/02.

Brewer, R. 1988. The Science of Ecology. W.B. Saunders Company. 921 pp.

Carvalho, G.R., and L. Hauser. 1994. Molecular genetics and the stock concept in fisheries. Rev. Fish Biol. Fish. 4: 326-350.

Cairns, D., K. Robichaud-LeBlanc, R. Bradford, R.H. Peterson, and R. Angus. 1999. Striped bass culture in eastern Canada. Department of Fisheries and Oceans Canadian Stock Assessment Secretariat Res. Doc. 99/04.

Cronin, M. A., W. J. Spearman, R. L. Wilmot, J. C. Patton, and J. W. Birkham. 1993. Mitochondrial DNA variation in chinook (*Oncorhynchus tshawytscha*) and chum salmon (*O. keta*) detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. Can. J. Fish. Aquat. Sci. 50: 708-715.

Diaz, M., G. M. Leclerc, and B. Ely. 1997. Nuclear DNA markers reveal low levels of genetic divergence among Atlantic and Gulf of Mexico populations of striped bass. Trans. Amer. Fish. Soc. 126: 163-165.

Hogans, W., and G. Melvin. 1984. Kouchibouguac National Park striped bass (*Morone saxatilis* Walbaum) fishery survey. Aquatic Industries Ltd. St. Andrews, NB, 91 pp.

Jessop, B. M. 1991. The history of the striped bass fishery in the Bay of Fundy. In Proceedings of a Workshop on Biology and Culture of Striped *Bass (Morone saxatilis*). Edited by R. H. Peterson. Can. Tech. Rep. Fish. Aquat. Sci. 1832. pp. 13-21.

Milner, G.B., D.J. Teel, F.M. Utter, and G.A. Winans. 1985. A genetic method of stock identification in mixed populations of Pacific salmon, *Oncorhynchus spp*. Marine Fisheries Review. 47: 1-8.

Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbour Sym. Quant. Biol. 51: 263-73.

Murphy, B. R., and D. W. Willis, editors. 1996. Fisheries Techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Nei, M. and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics. 105: 207-217.

O'Connell, M., and J. M. Wright. 1997. Microsatellite DNA in fishes. Rev. Fish. Bio. 7: 331-363.

Ohno, S. 1974. Chordata 1: Protochordata, Cyclostomata, and Pisces. Animal Cytogenetics. 4: 1-92.

Pamilo, P., and M. Nei. 1988. Relationships between gene trees and species trees. Mol. Biol. Evol. 5: 568-583.

Park, L. K., and P. Moran. 1994. Developments in molecular genetic techniques in fisheries. Rev. Fish. Bio. 4: 272-299.

Robichaud-LeBlanc, K. A., S. C. Courtenay, and A. Locke. 1996. Spawning and early life history of a northern population of striped bass (*Morone saxatilis*) in the Miramichi River estuary, Gulf of St. Lawrence. Can. JJZool. 74: 1645-1655.

Robinson, M., G. Klassen, A. Locke, A. Verschoor, E. Tremblay, A. St. Hilaire, and S. C. Courtenay. 1998. A preliminary survey of the early life history of striped bass (*Morone saxatilis*) in the Kouchibouguac Estuary in 1996. Tech. Rep. Eco. Sci. No. 12: 38 pp.

Rulifson, R. A., S. A. McKenna, and M. L. Gallagher. 1987. Tagging studies of striped bass and river herring in upper Bay of Fundy. Nova Scotia. ICMR Tech. Rep. 87-02, East Carolina University, Greenville, NC.

Rulifson, R. A. , and M. J. Dadswell. 1995. Life history and population characteristics of striped bass in Atlantic Canada. Trans. Amer. Fish. Soc. 114: 244-249.

Scott, W. B., and M. G. Scott. 1988. Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. 219: 731 p.

Stellwag, E. J., and R. F. Rulifson. 1995. Mitochondrial DNA stability and striped bass stock identification: Response to comment. Trans. Amer. Fish. Soc. 119: 956-959.

Utter, F., and Ryman, N. 1993. Genetic markers and mixed stock fisheries. Fisheries (Bethesda). 18 (8): 11-21.

Waldman, J. R., and I. I. Wirgin. 1995. Comment: mitochondrial DNA stability and striped bass stock identification. Trans. Amer. Fish. Soc. 124: 954-956.

Waldman, J. R., D. J. Dunning, Q. E. Ross, and M. T. Mattson. 1990. Range dynamics of

Hudson River striped bass along the Atlantic coast. Trans. Am. Fish. Soc. 119: 910-919.

Wirgin, I. I., R. Proenca, and J. Groosfield. 1989. Mitochondrial DNA diversity among populations of striped bass in the southeastern United States. Can. J. Zool. 67: 891-907.

Wirgin, I. I., P. Silverstein, and J. Groosfield. 1990. Restriction endonuclease analysis of striped bass mitochondrial DNA: the Atlantic coastal migratory stock. Am. Fish. Soc. Symp. 7: 475-491.

Wirgin, I. I., and L. Meceda. 1991. Development and use of striped bass specific RFLP probes. J. Fish. Bio. 39 (Supplement A): 159-167.

Wirgin, I., and J. R. Waldman. 1994. What DNA can do for you. Fisheries, 19 (7): 16-26.

Wirgin, I. I., T. Ong, L. Maceda, J. R. Waldman, D. Moore, and S. C. Courtenay. 1993a. Mitochondrial DNA variation in striped bass (*Morone saxatilis*) from Canadian rivers. Can. J. Fish. Aquat. Sci. 50: 80-87.

Wirgin, I. I., T. Ong, L. Maceda, J. R. Waldman, and Crittenden, R.N. 1993b. Use of mitochondrial DNA polymorphisms to estimate the relative contributions of the Hudson River and Chesapeake Bay striped bass stocks to the mixed fishery on the Atlantic coast. Trans. Am. Fish. Soc. 122: 669-684.

Wirgin, I. I., B. Jessop, S. C. Courtenay, M. Pederson, S. Maceda, and J. R. Waldman. 1995. Mixed-stock analysis of striped bass in two rivers of the Bay of Fundy as revealed by mitochondrial DNA. Can. J. Fish. Aquat. Sci. 52: 961-970.

Wright, J. M. 1993. DNA fingerprinting of fishes. In Hochachka, P. and Mommsen, T. eds. Biochemistry and Molecular Biology of Fishes, Vol. 2. Elsevier, pp. 57-91.

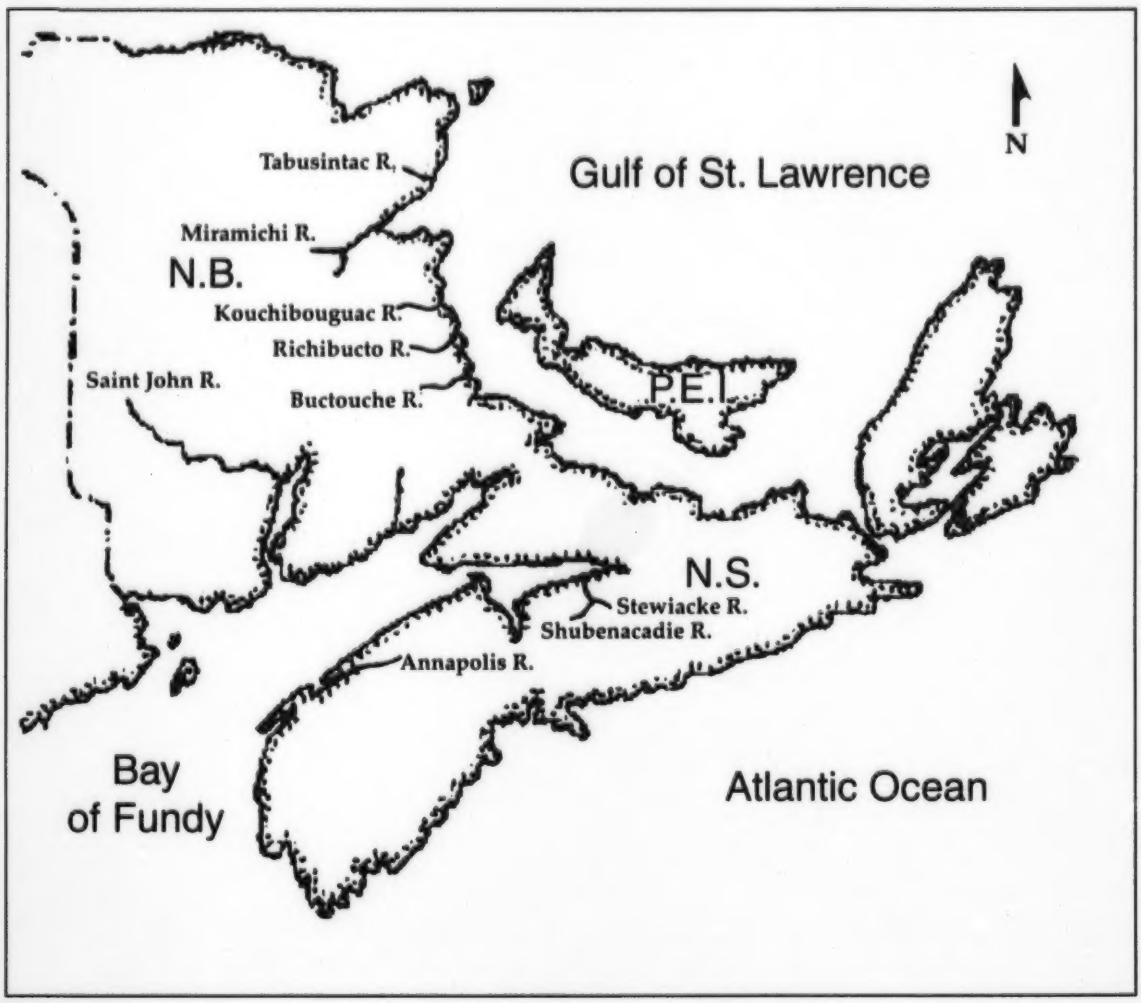

Figure 1: Map of the Maritime Provinces illustrating the Bay of Fundy, the southern Gulf of St. Lawrence, and the rivers mentioned in the text.

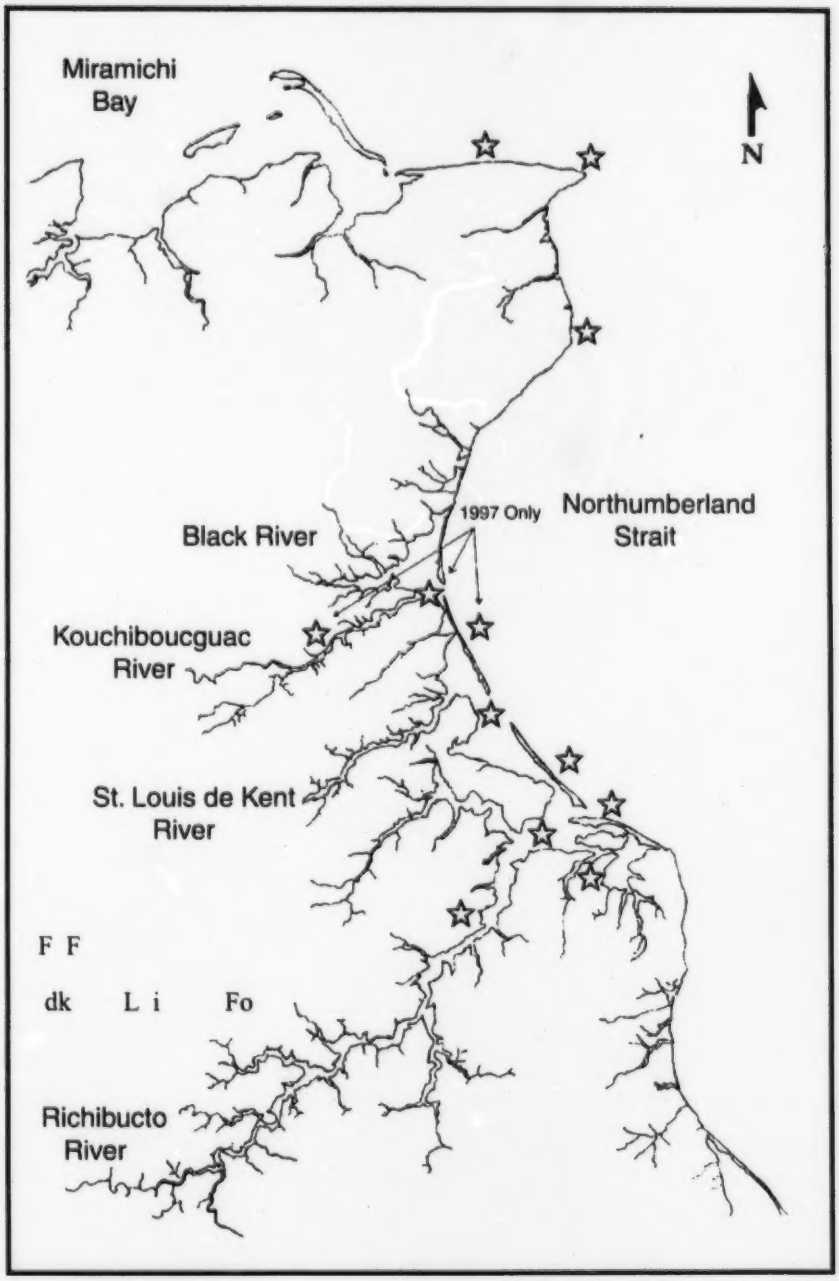

Figure 2: Map of the southern Gulf of St. Lawrence showing capture locations of YOY striped bass in 1997 and 1998.